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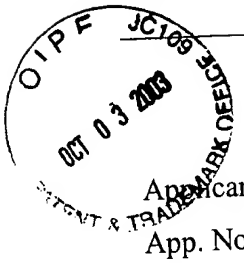

TRANSMITTAL

Applicant	:	Rousseau, et al.
App. No.	:	09/763,535
Filed	:	July 2, 2001
For	:	PHARMACEUTICAL COMPOSITION FOR TREATING OR PREVENTING DIABETES OR CANCER, OR THE WAARDENBURG SYNDROME
Examiner	:	D.M. Sullivan
Art Unit	:	1636

CERTIFICATE OF MAILING

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on

October 1, 2003
(Date)

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Sir:

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(X) Certified translation of claimed priority document in 11 pages.

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CERTIFICATION OF TRANSLATION

"PHARMACEUTICAL COMPOSITION FOR THE TREATMENT OR PREVENTION OF DIABETES OR CANCER"

I, Jennifer KILDEE,
c/o Technisches Fachübersetzungsbüro, Försterweg 33, A-2136 Laa/Thaya, Austria,
am the translator of the documents attached and certify that the following is a true translation to the best of my knowledge and belief.

Jennifer Kildee
Signature of translator dated this 15th day of September 2003

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PHARMACEUTICAL COMPOSITION FOR THE TREATMENT OR PREVENTION OF DIABETES OR CANCER

OBJECT OF THE INVENTION

The present invention relates to a novel pharmaceutical composition for the treatment or prevention of diabetes or cancer, in particular a cellular therapy for diabetes through the creation of an artificial pancreas.

TECHNOLOGICAL BACKGROUND OF THE BASIS OF THE INVENTION

Diabetes is a generic term under which are designated disorders characterized by a combination of polyuria and polydipsia. Diabetes mellitus, hereinafter also called sugar diabetes, which may be of a type 1 or type 2, is due to a poor functioning of the beta cells of the endocrine pancreas (islets of Langerhans) which synthesizes and secretes insulin (Gerich & Haeften, COED 5, pp. 144-148 (1998)). It is often accompanied (type 2 diabetes) by a resistance of the shock tissues to the action of insulin.

Sugar diabetes is one of the most common metabolic disorders, particularly in the industrialized world (Leahy, COED 5, pp. 73-74 (1998)). It is characterized by a deficiency in the utilization of glucose and may have serious and sometimes fatal pathological consequences, such as metabolic, cardiovascular and neurological problems, and lesions to the retina or kidneys. Treatment by insulin requires one or more daily injections for life.

Consequently, a definite need exists for replacing these injections with transplantable systems (Gage et al., *Nature* 392, Supplement 3 (1998)).

PRIOR ART

The document Lemaigre et al. (1996) describes a cDNA encoding the hepatocyte nuclear factor-6, hereinafter called HNF-6. This protein controls the transcription of certain genes in a small number of tissues where it is expressed (Samadani & Costa (1996)). The expression of this molecule was particularly identified in the pancreas of mice (Landry et al. (1997) and Rausa et al. (1997)).

The HNF-6 protein contains two domains for binding to DNA, one domain called cut and one domain called homeo, characteristic of HNF-6 by the presence of a phenylaline in position 48 and a methionine in position 50 (hereinafter called F48M50 dyad).

French Patent Application FR-2,696,755 describes an implantable capsule comprising an external envelope consisting of a hydrogel of acrylonitrile and sodium methallylsulfonate, an internal center comprising an encapsulated substance, which may consist of islets of Langerhans, beta pancreatic cells, or hepatocytes. The envelope is a biocompatible membrane selectively permeable to insulin or to the nutrients necessary to the encapsulated substance. This product may be utilized in the transplantation of cells or groups of cells such as islets of Langerhans for mitigating the insufficient production of insulin in diabetic patients.

International Patent Application WO95/09231 describes novel insulin-secreting beta cell lines which may appear in the form of "pseudoislets" and may be encapsulated in a biocompatible hydrogel; and possibly incorporated in the transplantable fibers intended for subcutaneous or intraperitoneal introduction in the patient for treating insulin-dependent patients.

International Patent Application WO95/29988 describes a procedure for culturing cell lines; particularly pancreatic cells, likely to create in vivo reimplantable cell islets in mammals so as to treat pancreatic illnesses in humans or animals.

GOALS OF THE INVENTION

The present invention aims to provide a novel pharmaceutical composition designed to be utilized in the prevention or treatment of diabetes or cancer and may be utilized either in the field of genetic therapy, or in the field of cellular therapy, by producing cellular masses or forming an artificial pancreatic tissue or organ.

CHARACTERISTIC ELEMENTS OF THE INVENTION

The inventors unexpectedly discovered that the invalidation of the HNF-6 gene in mice shows that this gene is essential for the function of islets of Langerhans and for the insulin response of the organism. Furthermore, the inventors have shown that other proteins similar to HNF-6, which share two of HNF-6's special features, firstly the presence of a single cut domain and secondly the presence of the F48M50 dyad in the homeo domain (Lannoy et al. (1998)) belong to the same ONECUT family (summarized in *OC*) (Lannoy et al. (1998)), also involved in certain essential metabolic mechanisms. Among the protein family thus defined, which includes the HNF-6 protein and the OC-2 protein among others, certain proteins have essential functions in animals, particularly in humans, particularly in glucose metabolism. Furthermore, such molecules may be utilized in treating a certain number of conditions and diseases, particularly diabetes or cancer, and preferably melanoma.

The present invention thus relates to a pharmaceutical composition comprising an appropriate pharmaceutical vehicle and an element chosen from the group consisting of a nucleotide sequence encoding a ONECUT family member protein, particularly HNF-6 or the OC-2 factor wherein the nucleotide and peptide sequence is hereinafter described (Figures 1a and 1b), a vector comprising the said nucleotide sequence, the encoded polypeptide sequence and/or a cell line transformed by the said vector and expressing these said nucleotide sequences, particularly likely to synthesize HNF-6 or another member of the ONECUT family such as the OC-2 factor.

"Nucleotide sequence encoding HNF-6" is understood to be the encoding sequence corresponding to the cDNA HNF-6 sequence such as already described, particularly by Lemaigre et al. (1996), and the equivalent human or animal sequences likely to hybridize with this cDNA. This hybridization is preferably carried out under stringent conditions so as to identify the different genomic sequences encoding a sequence of amino acids identical or similar to that of HNF-6 or OC-2. Particularly, these are other specific sequences from other mammals that have the same function, but are different particularly as regards redundancy of the genetic code. The standard hybridization conditions are preferably the following: hybridization at 40 °C in 50 % of formamide, 5 x SSC 20 mM sodium phosphate, pH 6.8, cleaning in 0.2 x SSC at 50 °C. Modifications to these conditions according to the length and content of GC nucleotides in the

sequence to be hybridized may be proposed by a person skilled in the art. Other hybridization conditions are, among others, those described by Sambrook et al., §§ 9.47-9.51 in *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, Laboratory Press, Cold Spring Harbor, New York (1989).

According to the invention, the gene encoding HNF-6 utilized concerns genomic sequences encoding the two alpha and beta isoforms of HNF-6 such as described by Lannoy et al. (1998).

The pharmaceutical composition of the invention may be utilized for obtaining a genetic and/or cellular therapy for a patient at risk for developing diabetes or suffering from diabetes, or at risk for developing cancer or suffering from cancer, particularly melanoma. In the genetic therapy field, the nucleotide sequence of the invention may be administrated to the patient or to the cell lines of the patient by a standard ex vivo treatment by procedures well known to a person skilled in the art or through a vector, preferably chosen from among the group consisting of plasmids, viruses, phagemids, and lipid vesicles such as cationic lipids, liposomes or a mixture thereof. The vector will incorporate all the elements necessary for obtaining the expression of the nucleotide sequence according to the invention in the patient, preferably in the specific cell lines to be treated, such as the pancreatic cells involved in the synthesis of insulin, hepatic cells involved in insulin response or epidermal cells or dermal cells at risk for developing melanoma.

The pharmaceutical composition of the inventor may also be utilized in cellular therapy by directly injecting cells in an in vivo or ex vivo procedure or by forming an artificial cell mass such as described in Patent Applications FR-2,696,755, WO95/09231 and WO95/29988. It is possible to obtain proliferation of the cells transformed by the nucleotide sequence of the invention or by the vector of the invention by procedures well known to a person skilled in the art, in particular those described in Patent Applications WO97/49728 and WO95/29988.

The pharmaceutical vehicle according to the invention varies according to the delivery system chosen (intravenous, intramuscular, oral, etc.) and is an excipient well known to a person skilled in the art, appearing in the form of tablets, pills, capsules, solutions, syrups, etc. This component may possibly comprise adjuvants (particularly a growth

hormone) well known to a person skilled in the art so as to introduce synergistic effects or to suppress certain specific cellular or immune reactions so as to reduce certain undesirable side effects or toxic effects from the active ingredient or vehicle of the invention.

The percentage of the active ingredient (nucleotide sequence, amino acid sequence or fragments thereof, vector, cell line, etc.) in the pharmaceutical composition may vary according to a very wide range, uniquely limited by the frequency of administration, tolerance to and level of acceptance of the composition according to the invention by the patient.

The present invention also concerns the utilization of the pharmaceutical composition of the invention in preparing medications intended for the treatment and/or prevention of type 1 or type 2 diabetes, conditions associated with diabetes, particularly conditions associated with the poor function of endocrine pancreas beta cells which synthesize and secrete insulin, and/or for the treatment of cancer, particularly melanoma.

A final aspect of the present invention concerns the method of treating the patient, particularly a patient at risk for developing diabetes, suffering from diabetes or at risk for developing cancer or suffering from cancer, particularly melanoma, for which the pharmaceutical composition of the invention is administered to said patient by an in vivo or ex vivo method of treatment.

REFERENCES

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Lemaigre, F.P. et al., Hepatocyte nuclear factor-6, a transcription factor that contains a novel type of homeodomain and a single cut domain. *Proc. Natl. Acad. Sci. USA.* 93, pp. 9460-9464 (1996)

Rausa, F. et al., The cut-homeodomain transcriptional activator HNF-6 is coexpressed with its target gene HNF-3b in the developing murine liver and pancreas. *Develop. Biol.* 192, pp. 228-246 (1997)

Samadani, U. & Costa, R.H., The transcriptional activator hepatocyte nuclear factor-6 regulates liver gene expression. *Mol. Cell. Biol.* 16, pp. 6273-6284 (1996)

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CLAIMS

1. A pharmaceutical composition comprising an appropriate pharmaceutical vehicle and an element chosen from among the group consisting of a nucleotide sequence encoding a peptide from the ONECUT family, a vector comprising this nucleotide sequence, the polypeptide sequence encoded by this nucleotide sequence and/or a cell line transformed by the said vector and expressing the peptide from the ONECUT family.

2. The pharmaceutical composition according to claim 1, characterized in that the peptide of the family is HNF-6.

3. The pharmaceutical composition according to claim 1, characterized in that the peptide of the family is OC-2.

4. The pharmaceutical composition according to any one of the previous claims, characterized in that the aforementioned nucleotide and polypeptide sequences are human nucleotide and polypeptide sequences.

5. The pharmaceutical composition according to any one of the previous claims, characterized in that the vector is chosen from among the group consisting of plasmids, viruses, phagemids, lipidic vesicles, particularly cationic vesicles, liposomes or a mixture thereof.

6. The utilization of the pharmaceutical composition according to any one of the previous claims for the preparation of a medication intended for the prevention and/or treatment of type 1 or type 2 diabetes or the conditions associated with diabetes, and to the prevention and/or treatment of cancer, particularly melanoma.

7. The therapeutic procedure for treating the patient, preferably a human patient at risk for developing or suffering from diabetes or cancer, particularly melanoma, characterized in that the pharmaceutical composition according to any one of claims 1 to 3 is administered ex vivo by isolating a bodily fluid or one or more cells from the patient, by treating the said cells or the cells present in this bodily

fluid by a vector of the invention, and by reinjecting the transformed cells into said patient.

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OC-2 Sequence

```
CCCCCGCCCGCCCCGGGCCCTGATGGACTGAATGAAGGCTGCCTACACCGCCTATCGATGCCTCACCAA 69

AGACCTAGAACGTGCGCCATGAACCCGGAGCTGACAATGGAAAGTCTGGGCACTTTGCACGGCGCGCGC 137
                    M  N  P  E  L  T  M  E  S  L  G  T  L  H  G  A  R  17

GGCGGCGGCAGTGGCGGGGGCGGCGGCGGGGGCGGCGGGGGCGGCGGCGGGGGCCCGGGCCATGAGCAG 207
 G  G  G  S  G  G  G  G  G  G  G  G  G  G  G  G  G  P  G  H  E  Q  40

GAGCTGCTGGCCAGCCCCAGCCCCCACCACGCGCGCCGCGGCCCGCGTGGCTCGCTGCGGGGCCCTCCG 276
 E  L  L  A  S  P  S  P  H  H  A  R  R  G  P  R  G  S  L  R  G  P  P  63

CCGCCTCCAACCGCGCACCAGGAGCTGGGCACGGCGGCAGCGGCGGCAGCGGCGGCGTCGCGCTCGGCC 345
 P  P  P  T  A  H  Q  E  L  G  T  A  A  A  A  A  A  A  A  S  R  S  A  86

ATGGTCACCAGCATGGCCTCGATCCTGGACGGCGGCGACTACCGGCCCGAGCTCTCCATCCCGCTGCAC 414
 M  V  T  S  M  A  S  I  L  D  G  G  D  Y  R  P  E  L  S  I  P  L  H  109

CACGCCATGAGCATGTCCTGCGACTCGTCTCCGCCTGGCATGGGCATGAGCAACACCTACACCACGCTG 484
 H  A  M  S  M  S  C  D  S  S  P  P  G  M  G  M  S  N  T  Y  T  T  L  132

ACACCGCTCCAGCCGCTGCCACCCATCTCCACCGTGTCTGACAAGTTCCACCACCCTCACCCGCACCAC 552
 T  P  L  Q  P  L  P  P  I  S  T  V  S  D  K  F  H  H  P  H  P  H  H  155

CATCCGCACCACCACCACCACCACCACCACCAGCGCCTGTCCGGCAACGTCAGCGGCAGCTTCACCCTC 621
 H  P  H  H  H  H  H  H  H  H  Q  R  L  S  G  N  V  S  G  S  F  T  L  178

ATGCGCGACGAGCGCGGGCTCCCGGCCATGAACAACCTCTACAGTCCCTACAAGGAGATGCCCGGCATG 690
 M  R  D  E  R  G  L  P  A  M  N  N  L  Y  S  P  Y  K  E  M  P  G  M  201
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Figure 1

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OC-2 Sequence (CONTINUED)

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AGCCAGAGCCTGTCCCCGCTGGCCGCCACGCCGCTGGGCAACGGGCTAGGCGGCCTCCACAACGCGCAG 759
 S  Q  S  L  S  P  L  A  A  T  P  L  G  N  G  L  G  G  L  H  N  A  Q  224

CAGAGTCTGCCCAACTACGGTCCGCCGGGCCACGACAAAATGCTCAGCCCCAACTTCGACGCGCACCAC 828
 Q  S  L  P  N  Y  G  P  P  G  H  D  K  M  L  S  P  N  F  D  A  H  H  247

ACTGCCATGCTGACCCGCGGTGAGCAACACCTGTCCCGCGGCCTGGGCACCCCACCTGCGGCCATGATG 897
 T  A  M  L  T  R  G  E  Q  H  L  S  R  G  L  G  T  P  P  A  A  M  M  270

TCGCACCTGAACGGCCTGCACCACCCGGGCCACACTCAGTCTCACGGGCCGGTGCTGGCACCCAGTCGC 966
 S  H  L  N  G  L  H  H  P  G  H  T  Q  S  H  G  P  V  L  A  P  S  R  293

GAGCGGCCACCCTCGTCCTCATCGGGCTCGCAGGTGGCCACGTCGGGCCAGCTGGAAGAAATCAACACC 1035
 E  R  P  P  S  S  S  S  G  S  Q  V  A  T  S  G  Q  L  E  E  I  N  T  316

AAAGAGGTGGCCCAGCGCATCACAGCGGAGCTGAAGCGCTACAGTATCCCCCAGGCGATCTTTGCGCAG 1104
 K  E  V  A  Q  R  I  T  A  E  L  K  R  Y  S  I  P  Q  A  I  F  A  Q  339

AGGGTGCTGTGCCGGTCTCAGGGGACTCTCTCCGACCTGCTCCGGAATCCAAAACCGTGGAGTAAACTC 1173
 R  V  L  C  R  S  Q  G  T  L  S  D  L  L  R  N  P  K  P  W  S  K  L  362

AAATCTGGCAGGGAGACCTTCCGCAGGATGTGGAAGTGGCTTCAGGAGCCCGAGTTCCAGCGCATGTCC 1242
 K  S  G  R  E  T  F  R  R  M  W  K  W  L  Q  E  P  E  F  Q  R  M  S  385

GCCTTACGCCTGGCAGCGTGCAAACGCAAAGAGCAAGAACCAAACAAAGACAGGAACAATTCCCAGAAG 1311
 A  L  R  L  A  A  C  K  R  K  E  Q  E  P  N  K  D  R  N  N  S  Q  K  408

AAGTCCCGCCTGGTGTTCACTGACCTCCAACGCCGAACACTCTTCGCCATCTTCAAGGAGAACAAACGC 1380
 K  S  R  L  V  F  T  D  L  Q  R  R  T  L  F  A  I  F  K  E  N  K  R  431

CCGTCAAAGGAGATGCAGATCACCATTTCCCAGCAGCTGGGCCTGGAGCTCACAACCGTCAGCAACTTC 1449
 P  S  K  E  M  Q  I  T  I  S  Q  Q  L  G  L  E  L  T  T  V  S  N  F  454

TTCATGAACGCCCGGCGCCGCAGCCTGGAGAAGTGGCAAGACGATCTGAGCACAGGGGGCTCCTCGTCC 1518
 F  M  N  A  R  R  R  S  L  E  K  W  Q  D  D  L  S  T  G  G  S  S  S  477

ACCTCCAGCACGTGTACCAAAGCATGATGGAAGGACTCTCACTTGGGCACAAGTCACCTCCAAATGAGG 1587
 T  S  S  T  C  T  K  A
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Figure 1b